

Studies on some parametric variables for ethanol production

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Abstract : The present communication deals with the parametric variables for ethanol production by *Saccharomyces cerevisiae* NCIM -2086. This strain was subjected to parametric studies. Major effects were caused due to pH value, incubation temperature. Incubation period and concentration of the blackstrap molasses. It has been reported that optimum values for alcoholic fermentation has been found when blackstrap molasses 16% (w/v) is allowed to ferment for 47 hours at 31°C by maintaining the pH value of the fermentation medium at 4.9 in the presence of yeast *Saccharomyces cerevisiae* NCIM -2086.

(Keywords : Alcoholic fermentation, *Saccharomyces cerevisiae* NCIM -2086, molasses)

Introduction.

Yeasts are cosmopolitan microorganisms that are mostly found in natural ecosystems rich in sugar¹. Mohd Azhar *et al.*² estimated that over 150, 0000 yeast species are distributed on earth of which only 1%.

Plants are the preferred habitats for yeasts and they include nectars, flowers, fruits, decaying tissues, and tree saps^{3,4}. These plant parts also attract insects that not only help the distribution of yeasts to different habitats but also play a great role in diversifying the yeast communities in flowers making the yeast diversity seasonal since the pollinating insects are mostly seasonal^{4, 5, 6}. In addition, yeasts are widely distributed in the soil¹.

Traditionally, yeasts from the genus *Saccharomyces* are used in bakery and brewery industries for ethanol production of high spirit and

industrial grade ethanol for human consumption from simple sugars. However, simple sugars are relatively expensive substrates for economical ethanol production. On the other hand, ethanol can also be produced from agricultural crush (starch and lignocellulose), sugar industry wastes (sugar cane and beet molasses), and dairy industry waste (whey) with a dual purpose of producing energy from cheap sources and alleviation of environmental pollution⁹.

It is well established that yeast growth and ethanol production are influenced by different nutritional and environmental factors such as temperature, pH, oxygen, and initial sugar concentrations^{10,12,14}. Temperature and pH affect membrane turgidity, enzymatic activity and metabolism of yeast cells¹³. They usually prefer acidic pH that enables to control bacterial growth at industrial level. Consequently, yeasts which are active and tolerant to high temperature and low pH are ideal for industrial bioethanol production.

Ethanol fermentation was one of the first chemical reactions observed by humans. Parametric studies of different strains causing alcoholic fermentation is done either by classical or statistical techniques¹⁻⁷. Fermentation media for alcoholic fermentation should consist of substrate necessary for the growth of microorganisms, primarily the carbon, nitrogen and phosphorus sources. Moreover, water and air can be included as fermentation substrate sources⁸⁻⁹. The basic substrates for alcoholic fermentation using submerged technique of fermentation are beet or cane-molasses¹⁰. The present investigation deals with parametric studies of alcoholic fermentation.

Blackstrap molasses was employed as the basal fermentation media in the fermentor flasks under the submerged fermentation conditions. The study reveals the nutritional status of the organisms and basic fermentation parameters.

Experimental

Medium :

The composition of production medium for ethanolic fermentation by *Saccharomyces cerevisiae* NCIM -2086 is prepared as under :
Molasses :16% (w/v), Malt-Extract : 1.25%
Yeast-Extract : 1.25%, Peptone :1.25%
Distilled water : To make up 100 ml, pH: 4.9

Culture medium

Saccharomyces cerevisiae -2086 was periodically cultured on malt-agar media. The fresh culture media was prepared every fortnight as follows : Sucrose : 8%, Malt extract : 0.30g
Yeast extract : 0.30g, Peptone : 0.40 g , Agar - Agar: 0.30g, Distilled water : 100 ml, pH : 4.9

Sterilization :

The growth and production media were sterilized in an autoclave maintained at 15 lbs steam pressure for 30 min.

Strain : *Saccharomyces cerevisiae* - 2086 was used in the present study. The strain was procured from NCL, Pune, India.

Assay methods:

Evaluation of ethanol formed and molasses left unfermented was made colorimetrically¹³⁴⁻¹³⁵

Age of the inoculum: 47 hours old.

Quantum of the inoculum: 0.5 ml yeast suspension of *Saccharomyces cerevisiae* NCIM-2086

Molasses concentration : 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 20% and 25%.

Temperature (in°C) : 25, 27, 29, 31, 33, 35, 39, 43, 48 and 52°C

Incubation period: 15, 20, 22, 25, 47, 69, 73, 75, 80 and 81 hours

pH : 3.6, 3.8, 4.0, 4.2, 4.4, 4.6, 4.9, 5.0, 5.1 and 5.2.

Results and Discussion

The results obtained from optimization of different parameters show that optimum values for ethanolic fermentation by *Saccharomyces cerevisiae* NCIM -2086 proceeds best when 16% (w/v) molasses solution is allowed to ferment for 47 hours at 31°C by maintaining the pH value of the fermentation medium at 4.9 in the presence of strain of yeast *Saccharomyces cerevisiae* NCIM-2086.

The results of ethanolic fermentation by *Saccharomyces cerevisiae* NCIM -2086 in the present investigation support the view that in general ethanolic fermentation by *Saccharomyces cerevisiae* NCIM -2086

Table - 1
Effect of different substrates on ethanolic fermentation by *S. cerevisiae* NCIM -2086 after 47 hours of incubation period

S. No.	Carbohydrates	Yield of ethanol* ml/100 ml	Substrate left unfermented in g/100ml
1.	Arabihose	1.33	—
2.	Rhamnose	1.18	—
3.	Xylose	0.75	—
4.	Glucose	5.90	2.489
5.	Fructose	5.39	2.632
6.	Galactose	4.30	3.318
7.	Sorbose	1.46	—
8.	Lactose	3.66	—
9.	Sucrose	6.15	2.563
10.	Maltose	1.39	—
11.	Starch	0.86	—
12.	Inulin	****	—
13.	Dextrine	****	—
14.	Raffinose	****	—
15.	Mannitol	****	—
16.	Molasses	4.35	2.906

* Each value represents mean of three trials

** Optimum values of molasses solution, pH, temp. and incubation period. *** Optimum yield of ethanol. **** Insignificant value.

becomes more easier as the size, molecular weight and the structure complexity of the sugar molecules decreases. It is obvious that the simplest sugars glucose and fructose both are easily fermentable because both the monosaccharide sugars are easily phosphorylated. Galactose fermentability is also much similar to glucose and fructose while arabinose, rhamnose and sorbose were found to be least fermentable.

Sucrose is made from glucose and fructose units. Sucrose a disaccharide sugar has been found suitable for production of ethanol while

lactose was found to be unsuitable. However, maltose was fermented to some extent on the basis of total sugar taken. In the group of polysaccharides, starch was found to be least fermentable and unsuitable for ethanolic fermentation. However, raffinose, inulin and dextrin produced no ethanol. Polyethanol mannitol also could not produce any ethanol by fermentation. On the basis of above observation it is concluded that glucose amongst monosaccharides and sucrose amongst disaccharides are most suitable and useful for ethanolic fermentation.

Table -2
Effect of concentration of molasses, pH, temperature and incubation period on ethanolic fermentation by *Saccharomyces cerevisiae* NCIM -2086.

% of molasses	pH	Temp. in (°C)	Incubation period in hours	Corresponding yield of ethanol* in ml/100 ml			
2	3.6	25	15	0.45	2.08	3.15	0.85
4	3.8	27	20	1.50	2.15	3.25	1.30
6	4.0	29	22	1.98	2.45	3.86	1.40
8	4.2	31**	25	2.80	2.80	4.16***	1.98
10	4.4	33	47**	3.15	3.15	4.06	4.18**
12	4.6	35	69	3.20	3.88	****	3.01
14	4.9**	39	73	3.52	4.35***	****	*****
16**	5.0	43	75	4.05***	4.15	****	****
20	5.1	48	80	3.90	****	****	****
25	5.2	52	85	3.75	****	****	****

* Each value represents mean of three trials ** Optimum values of molasses solution, pH, temp. and incubation period. *** Optimum yield of ethanol. **** Insignificant value.

References

1. R. Vadkertiová, H. Dudášová, E. Stratilová, M. Balašáková *Yeast*, **36** (10), 617 ((2019))
2. S.H. Mohd Azhar, R. Abdulla, S.A. Jambo, H. Marbawi, J.A. Gansau, A.A. Mohd Faik, K.F. *Biochemistry and Biophysics Reports*, **10**, 52 (2017)
3. A.H. Li, F.X. Yuan, M. Groenewald, K. Bensch, A.M. Yurkov, K. Li, P.J. Han, L.D. Guo, M.C. Aime, J.P. Sampaio, S. Jindamorakot, B. Turchetti, J. Inacio, B. Fungsin, Q.M. Wang, F.Y. Bai *Stud. Mycol.*, **96**, 17 (2020)
4. M. von Arx, A. Moore, G. Davidowitz, A.E. Arnold *PloS One*, **14** (12) (2019)
5. L.A.I. de Azeredo, E.A.T. Gomes, L.C. Mendonça-Hagler, A.N. Hagler *Int. Microbiol.*, **1** (3) 205 (2010)
6. A.M. Glushakova, A.V. Kachalkin, I.Y. Chernov *Microbiology*, **83** (1), 125 (2014)
7. M. Ashenafi Ethiop. *J. Biol. Sci.*, **5** (2), 189 (2006)

8. D. Sandoval-Nuñez, M. Arellano-Plaza, A. Gschaedler, J. Arrizon, L. Amaya-Delgado *Clean Technol. Environ. Policy*, **20** (7), 1491 (2018)
9. M.M. Manyuchi, P. Chiutsi, C. Mbohwa, E. Muzenda, T. Mutusva S. Afr. *J. Chem. Eng.*, **25**, 123 (2018)
10. P. Saini, A. Beniwal, A. Kokkiligadda, S. Vij *Process Biochem.*, **72**, 1 (2018)
11. N.A. Chohan, G.S. Aruwajoye, Y. Sewsynker-Sukai, E.B. Gueguim Kana *Renew. Energy*, **146**, 1031 (2020)
1. R. L. Mongahan, and L.R. Koupal, Novel microbial products for medicine and Agriculture Elsevier New-York, PP. 25-32 (1989).
2. M. D. Young, L. K. Kempe, and F. G. Bader *Biotechnol Bioeng.* **27**, 327 (1985).
3. G. Holt, G. Saunders, A. L. Demain, N. A. Solomon (eds) Manual of Industrial and Microbiology and Biotechnology, American Society of Microbiologists, Washington D.C. PP. 41-48 (1986).
4. D. W. Zabriskie, W. B. Armiser, D. A. Arbano and D. H. Phillips, Trader's guide to fermentation, media, Formulation Trader's Protein Mephis Tenn (1988).
5. M. R.S. Srinivas, Nagin Chand and B. K. Lonsane *Bioprocess Eng.*, **10**, 139 (1993).
6. R. L. Greasham, H. J., Reed, G., Puhler Rehm Biotechnology Vol. 3, Bioprocessing VCH Publishers A. and Stadler, P. (eds.) Inc., New-York, PP. 128-139 (1986)
7. R. L. Greasham and E. Inamine Manual of Industrial microbiology and Biotechnology American Society of Microbiologists, Washington, D. C. PP. 41-48 (1986)
8. S. P. Singh, U. N. Verma, H. K. K. Kishore and G. Samdani *Oritenal J. Chem.* **14**, 333 (1998)
9. I. Haque, S. Khurshid, S. Ali, H. Asharf, M.A. Quadeer and M. I. Rajoka *World Journal of Microbiology and Biotechnology* **17**, 35 (2001).
10. M. Pazouki, P. A. Felse, J. Sinha and T. Panda *Bioprocess Engineering* **22**, 353 (2000)
11. L.P. McCloskey and L. L. Replogle Am. J. Enol Vitie **25**, 194 (1974).
12. M. Dubois, K. S. Gills, J. K. Hamilton, P. A. Rebers and F. Smith *Anal Chem.* **28**, 350 (1956).
13. S. P. Singh, B. Kumar, B. Pratap, A. K. Pandey & S. K. Srivastava, Proc. VIII Carbohydrate Conf. Nov. 18-20, PP. 173-175 Trivandrum, (1992).
14. V. Krumphazal, D. Josef and J. Strejeck, Czech **116**, 443 (Cl. Cl12d) Oct. 15 1965, Appl June 29 pp-3 (1964).
15. S. P. Singh, B. Kumar, R. K. Pandey, and A. Bihari, *Biojournal*, **4** (1 & 2), 245 (1992).
16. S. P. Singh, A. P. Sinha, M. Prasad, L. Kumar, and S.K. Yadav, *Biojournal*, **4** (1 & 2), 241 (1992)
17. S. P. Singh, B. Pratap, B. K. Ambastha, R. K. Pandey, A. C. Sinha, and A. Prasad, *Asian J. Chem.* **6**, 753 (1994).
18. S. P. Singh, A. K. Brahmachari M. K. Singh, and K. P. Kamal, *Asian J. Chem.* **9**, 157 (1997).
19. S. P. Singh, B. K. Ambastha, and N. Rathor, *Asian J. Chem.* **10**, 375 (1998).
20. R. Kumar, C. Kumari, A. K. Nag, K. S. Sinha, Jyotimala and S. P. Singh, *J. Chemtracks* **10**(1&2), 375 (2008).